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ESTABLISHMENT OF AEROGENICALLY TRANSMITTED VIRUSES -- EXPERIMENTS ON SEDIMENTATION OF VIRUS IN AEROSOLS

Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene
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Jakob Petmezakis

Hospitalism, its causes and its control, confront scientists dealing with this topic with some rather difficult problems. The exploration of the manner in which viruses are spread, particularly the study of aerogenous transmission, is particularly important here.

At the International Symposium on Smallpox Vaccination, Lyon, 1962, MacCallum tried to discuss and clarify the problems of smallpox virus communication at the smallpox stations as such and in the closer and broader vicinity of hospitals; he thought that the escape of virus-containing aerosols through the windows plays a great role here. He quoted the investigations of Power (1885) who systematically checked 1,000 houses in the vicinity of a smallpox hospital and who discovered the typically graduated occurence of smallpox cases; the origin theories on the communication of this disease through the air are based on this. But not even MacCallum could be sure of his explanation as to the way in which smallpox can be communicated near hospitals or whether this was just an accidental infection. In his opinion, however, we can be sure that infectious aerosols do develop in smallpox wards and these aerosols can spread through the air. He indicated that very careful and thorough investigations are required to answer this question.

This problem became particularly important as a result of the outbreaks of smallpox in 1961 and 1962 in North Rhine-Westphalia (Lammersdorf-Simmerath, Kreis Monschau). In this connection, Anders (1963) reported that a 9-year old girl supposedly became sick due to infection through coughing because she was 20 meters away from sick persons inside the hospital.

It is my purpose here to investigate the dynamics of an infectious aerosol and to obtain a more accurate idea as to the mechanism of the aerogenous communication of virus-conditioned diseases. For this purpose I

performed experiments with virus-containing aerosols:

(A) in the droplet phase
(B) in the dust phase

considering various factors which are at work in the air and which may influence the aerosols.

Properties of Aerosols

The viruses in the air are found there in the form of aerosols and we find that the same physical-chemical laws apply to the microbial aerosol as to industrial aerosols.

By the term aerosols we mean colloid systems with gaseous dispersion means. Liquid substances distributed in a colloid fashion are fogs or mists whereas in the solid state they are various forms of dust. In an isodispersed aerosol, all components roughly have the same size; a polydispersed aerosol consists of particles of varying size. The suspended particles contained in an aerosol can vary in size. We distinguish coarse-dispersed ($> 10^{-5}$ cm diameter), colloid-dispersed (10^{-5} to 10^{-7} cm diameter), and molecular-dispersed systems ($< 10^{-7}$ cm diameter)(Carlson 1960-1961).

Compared to other colloid systems, aerosols generally contain a large proportion of coarse particles (Sirgenson and Straumanis, 1949). It is therefore difficult to get aerosols whose particles is less than 200 mmu. Condensation aerosols (that is, aerosols with condensed droplets) primarily reveal particles whose diameter is about 600 mmu but we can often also encounter particles with a size of about 20 mmu diameter.

The concentrations of the aerosols generally are between 1-1,000 mg/m^3. At higher concentrations, the particles cannot remain in suspension for a longer period of time because they coagulate and become sedimented. As a result of this sedimentation, the aerosols are converted into aerogels (gels result from the combination of tiny primary particles of a sol into larger secondary particles); this is a process which is important in aerosol separation. Stokes' resistance equation applies only for coarse dust particles and droplets with a diameter of more than 20 mu (see formula below, under subheading 2. Experiment).

The stability of an aerosol depends on various environmental conditions, such as, the electrical room charge, the air movement, the temperature differences, the degree of dilution, as well as the physical and chemical character of the dispersion agent (Kliewe and Wasielewski, 1952).

The physical and chemical properties of a dispersion agent can influence the aerosol; for instance, when we atomize non-albumen containing liquids, water evaporation is speeded up and the size "circumference" of the droplets is thus reduced (Gaidamovich and Vlodavets, 1963). In other words, virus particles can remain suspended in a non-albumen containing liquid for a lonter time and in larger concentrations than in albumen-containing liquids.

But there is reason to assume that the environmental factors (Brown's molecular movement, electrical room charge) have a greater influence on the stability of an aerosol than the properties of the dispersed portion. If we have an aerosol with extremely finely dispersed suspended particles, we can observe a relatively fast agglomeration of the particles, whereby we get an average of 2.5 mu for the diameter. If we get this value then the agglomeration stops when the corresponding aerosol is present in a high concentration (Remy, 1949).

Author's Investigations

Preliminary Experiments

I selected bacteriophages as model for my preliminary experiments because they have no pathogenicity, because their presence can be established quite readily, and because they require little in the way of preparation time. In my preliminary experiments I was primarily interested in the following:

1. The applicability of Koch's sedimentation method (R. Koch);
2. The duration of suspension;
3. The dependence of the sedimentation tendency on the air humidity in the experimentation chamber.

Material and Methods

For my preliminary experiments I used an airtight chamber with an air volume of 387 liters in which a T_3 bacteriophage suspension was atomized by means of a medication atomizer (Type MV-1, Drager Company, Lubeck).

The atomizer nozzle was connected to a compressed-air bottle from which gas flowed into a pressure-reducer through the bottle valve (see Figure 1). Here we can reduce the gas to a lower working pressure and it then flows in variable volumes through the immersion tube and the atomizer nozzle into the atomizer bottle. While passing through the nozzle, the gas sucks the phage-containing liquid in and atomizes it. The nozzle should produce mostly particles with a diameter of 1-5 mu (according to the Drager Company, Lubeck, the average particle diameter is around 2 mu); however, I did not work out a breakdown of the particle size here.

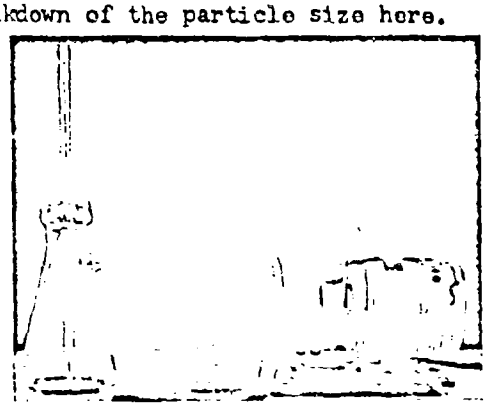

Fig 1. Suction bottle, connected to vacuum pump.

When we investigate the behavior of phage-containing aersols, we should use methods and apparatus which:

(a) will be easy and fast to handle;
(b) will be equally easily and well applicable to coarse and to small phage-containing, respectively, virus-containing dust particles;
(c) will guarantee the fastest possible registration and identification of all phages (respectively, vaccine-viruses) or phage-loaded particles per volume of air; and
(d) will facilitate the determination also of short-term changes in the phage or virus concentration.

The literature contains numerous methods which can be used for the establishment or counting of living microorganisms found in the air (Albrecht, 1955).

In addition to the methods mentioned by Albrecht (1955) optical methods have also been used to establish the presence of microorganisms in the air (Ferry, Farr and Hartman, 1949; Ferry, Farr, et al, 1951; Gucker and O'Konski, 1949). But these methods are disadvantageous in that it is very difficult to obtain a differentiation or specific identification of the microorganisms with them and in some cases this is impossible altogether.

Koch's sedimentation method is based on the principle that particles suspended in the air can be identified through the exposition or exposure of plates. Of course, this method, compared to the aspiration methods described under the heading of Main Experiments, is not sufficiently accurate; but it does offer the advantage that the atmosphere in the experimentation chamber is not "violated." I atomized 0.8 ml of a bouillon-diluted bacteriophage suspension containing 18,000 units per ml.

The air humidity was adjusted prior to each experiment by spraying air with a spray bottle; here I had to make sure that the water droplets, which for the time being were still necessary to produce air humidity, would be sedimented-out prior to the subsequent phage-atomization. The relative air humidity was controlled during the experiments with a hygrometer. To perform Koch's sedimentation experiment, I used Petri dishes which contained blue-agar inoculated with the coli strain "B". After determination of the experiment, the dishes were incubated for 24 hours in the incubator at a temperature of 37° C.

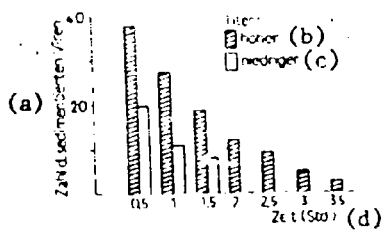

Fig 2. Comparison of sedimentation of a virus aerosol with high and low titer.
Key: a. number of viruses sedimented
b. high
c. low
d. time (hours)

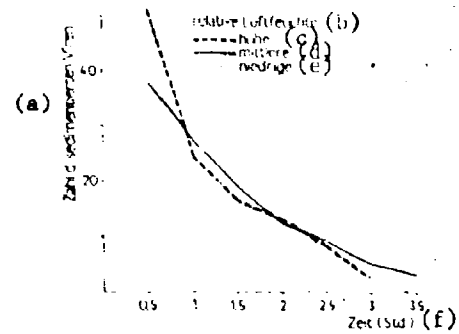

Fig 3. Dependence of sedimentation of a virus aerosol on relative air humidity.

Key: a. number of viruses sedimented
b. relative air humidity
c. high
d. medium
e. low
f. time (hours)

Results of Preliminary Experiments

1. Experiment for determination of suspension time, respectively, sedimentation tendency of a phage aerosol:

The prepared plates were exposed at 3 different points in the chamber (designated with I, II, and III in Table 1) for 10 minutes, each, at various times, more specifically, during atomization, right afterward, and then 30 minutes, 1 hour, 1-1/2 hours, 2 hours, and 2-1/2 hours after atomization.

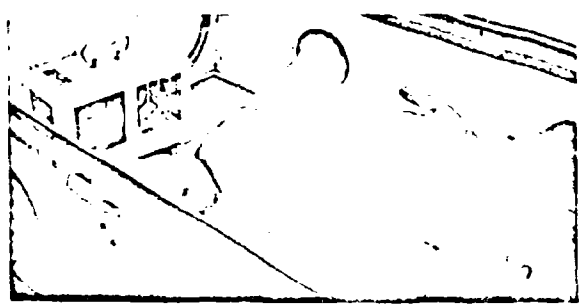

Fig 4. Introduction of Petri dishes, containing blue-agar and Coli B/am into the experimentation chamber.

Table 1. Suspension Time and Sedimentation Tendency of T_3 Phages

Exposure Time	Sampling Points	No of Phages Per Dish	Relative Humidity	Temperature
Prior to atomization, empty control		ø	50%	20° C
During atomization	I			
	II	+ +	53%	21° C
	III			
Right after atomization	I	30		
(10 min exposure)	II	25	54%	21.5° C
	III	23		
30 min after atomization	I	14		
(10 min exposure)	II	16	53%	21° C
	III	17		
1 hour after atomization	I	11		
(10 min exposure)	II	14	49%	21.2° C
	III	12		
1-1/2 hrs after atomization (10 min exposure)	I	7		
	II	4	49%	21° C
	III	3		
2 hrs after atomization	I	ø		
(10 min exposure)	II		50%	21.1° C
	III			
2-1/2 hrs after atomization (10 min exposure)	I			
	II	ø	50%	21° C
	III			

Symbols: ++ Phages cannot be counted
ø No phages

The relative humidity in the experimentation chamber corresponded to the humidity prevailing in areas or rooms on our geographic latitudes (40-50%); the temperature was about 20-22° C. The results obtained here are shown in Table 1; this table shows us that phages can still be established in the air 1-1/2 hours after atomization.

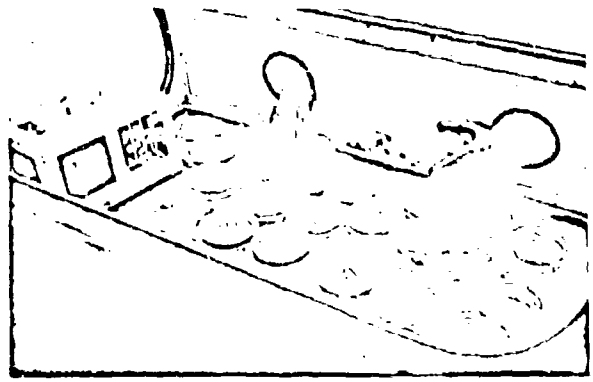

Fig 5. Opening and closing the Petri dishes during experiments; on the left, the hygrometer for measuring the humidity.

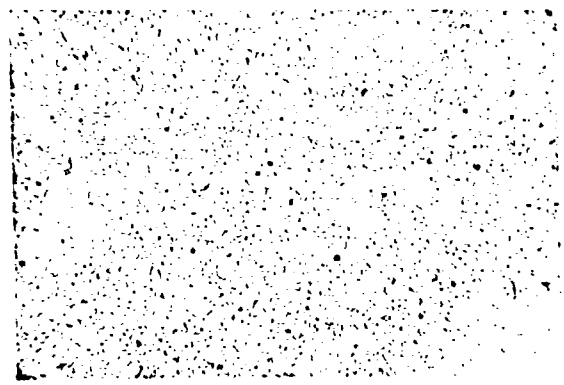

Fig 6. Compact FL cell growth used to establish the virus aerosol (microscopic photograph, enlarged 1:80).

2. Experiment for investigation of influence of reduced relative humidity:

To achieve a lower relative humidity, we placed two dishes with dry calcium chloride on the bottom of the experimentation chamber. Within 20 hours, the relative humidity dropped to 16-20% and this was enough for the determinations we wanted to make here. Table 2, below shows us the results achieved.

The number of phages established on the Petri dishes had been reduced in contrast to the numbers found at normal humidity; when the air humidity was lower, the phage concentration in the chamber also decreased somewhat more slowly.

Table 2. Sedimentation Tendency of T_3 Phage Aerosol at Reduced Relative Air Humidity

Zeit der Exposition (a)	Entnahmestellen (b)	Anzahl der Phagen pro Schale (c)	Relative Luftfeuchte (d)	Temperatur (e)
Vor Vernebelung (Leerkontrolle) (f)		ø	17%	22°C
Während der Vernebelung (g)	I II III	+	17%	22,2°C
Unmittelbar nach Vernebelung (h) (10 Min. Exposition)	I II III	20 17 18	17%	22,3°C
30 Minuten nach Vernebelung (i) (10 Min. Exposition)	I II III	11 14 10	20%	22,2°C
1 Stunde nach Vernebelung (j) (10 Min. Exposition)	I II III	7 6 8	20%	22,3°C
1,5 Stunden nach Vernebelung (k) (10 Min. Exposition)	I II III	4 3 2	20%	22,4°C
2 Stunden nach Vernebelung (l) (10 Min. Exposition)	I II III	ø	20%	22,6°C
2,5 Stunden nach Vernebelung (m) (10 Min. Exposition)	I II III	ø	18%	22,5°C

Key: [for Items a -- m, see Key, Table 1, above]
Symbols: + at the limit of countability
ø no phages

3. Experiment on the influence of increased relative air humidity on the suspension time of the T_3 phage aerosols:

The experimental setup here was the same as in experiments 1 and 2. To achieve the desired relative air humidity, we proceeded as described above (control by means of a hygrometer). Table 3 shows us that, in combination with high humidity, considerably more T_3 phage droplets were sedimented during the first 30 minutes than when the air humidity was medium or low. Subsequently, however, we observed a continual decrease in sedimentation. The large number of T_3 phages, sedimented during the first 30 minutes after atomization, caused a rapid decrease in the phage concentration and this, again, led to a shortening of the suspension time.

The results obtained in the preliminary experiments with respect to the influence of the suspension time and the relative humidity will be discussed together with the results of the main experiments at the end of this article.

Table 3. Sedimentation Tendency of T3 Phage Aerosol at Increased Relative Air Humidity

Zeit der Exposition (a)	Entnahmestellen (b)	Anzahl der Phagen pro 5 Liter (c)	Relative Luftfeuchte (d)	Temperatur (e)
Vor Vernebelung (Leerkontrolle) (f)		ø	88%	21°C
Während der Vernebelung (g)	I II III	+ +	90%	21,1°C
Unmittelbar nach Vernebelung (h) (10 Min. Exposition)	I II III	+	91%	21,2°C
30 Minuten nach Vernebelung (i) (10 Min. Exposition)	I II III	40 51 44	80%	21°C
1 Stunde nach Vernebelung (j) (10 Min. Exposition)	I II III	32 28 36	88%	20,8°C
1,5 Stunden nach Vernebelung (k) (10 Min. Exposition)	I II III	ø	87%	20,8°C
2 Stunden nach Vernebelung (l) (10 Min. Exposition)	I II III	ø	86%	20,8°C
2,5 Stunden nach Vernebelung (m) (10 Min. Exposition)	I II III	ø	80%	20,7°C

Key: [For items a -- m, see Legend, Table 1, above]
Symbols: ++ cannot be counted
+ at the countability limit
ø no phages

Main Experiments

A. Droplet Phase

Material and Methods

To carry out the main experiments, I used the experimentation chamber and the nozzle employed in the preliminary experiments. I sprayed a vaccine virus suspension which had been prepared as follows:

The suspension was obtained from calf raw material which had been ground up with buffered cooking salt solution and glycerine (2 g raw material + 2 ml NaCl + 6 ml glycerine). This suspension was diluted with Hanks-medium + LH (lactalbuminhydrolysate, yeast extract)(Hennebert and Kohler, 1961) and it was centrifuged at 3° C and 1,000 rpm. Repeated titration revealed values of about $5 \cdot 10^7$ units/ml.

There are two methods which turned out to be quite good in this phase of the experiment:

1. Koch's sedimentation method without measurement of air volume;
2. The aspiration method.

1. Koch's Sedimentation Method

To establish viruses by means of Koch's sedimentation method, cells of the FL strain were bred in plastic cups or dishes with a diameter of 5.5 cm. In order to achieve the formation of a compact cell carpet within an incubation time of 48 hours at 37° C, 6 ml of cell suspension were pipetted into each dish (140,000-160,000 cells per ml).

2. Aspiration Method

For this method we used the simplified "capillary impinger" according to Rosebury (1947) (see Figure 1). The apparatus consists of a suction bottle with a capacity of 500 ml, sealed by a perforated rubber stopper. In the perforation, we have a 1-mm pipette whose tip ends about 3-5 mm above the bottom of the suction bottle and dips into the washing liquid. The suction bottle is connected to a Pfeiffer vacuum pump. The volume of air to be transported depends on the diameter of the lower pipette opening, assuming that the suction force of the pump is constant; in the washing bottles used in this experiment, the air volume was between 9.5 and 10.0 l/min. The air volume was measured with a gas clock.

In the aspiration method, the particles in the air are expelled due to the high flow speed of the air in the pipette along the glass bottom and the water surface and they are enriched in the washing liquid.

In the experiments performed here, the washing liquid used was Hanks solution + LH, modified in the Robert Koch Institute (Hennoberg and Kohler, 1961). By adding silicon anti-foaming agent (SH), we tried to reduce the foaming of the Hanks solution and to cut down on the loss of fine water droplets, respectively, to prevent any coagulation of the viruses in the solution. We weighed the washing liquid before and after the experiment and found that the losses were quite minor. Besides, it was necessary to add antibiotics in order to eliminate any accidental bacteria-containing additions of air as much as possible.

Experiments

1. Experiment

To simplify the following experiments, the first thing we wanted to investigate was the distribution tendency of the aerosol. If it turned out that the distribution was uniform, then we would have to take samples at only one sampling point in the subsequent experiments in order to examine the ratios and conditions here.

The plates were exposed at 3 different places in the chamber (designated I, II, and III in Table 4) for 10 minutes, each, at various times, more specifically, during atomization (about 2 minutes), right afterward, and 30 minutes, 1 hour, 1-1/2 hours, 2 hours, 2-1/2 hours, 3 hours, and 3-1/2 hours after atomization. The relative air humidity in the experimentation chamber was 40-50% and the temperature was about 20-22° C.

We atomized 0.5 ml of virus suspension (titer: $5 \cdot 10^7$/ml) in 2 minutes, in other words, a total of 25 million viruses. If we assume that every drop constitutes a CPE (cytopathogenic effect) on the cell layer, then the experiments prove that the large drops caused big CPE areas on the first-exposed plates (during and right after atomization) because of their strong sedimentation tendency, whereas smaller droplets formed smaller CPE spots. The values obtained for the CPE and given in Table 4 show that the viruses were distributed uniformly throughout the chamber 30 minutes after atomization. The amount of the virus quantity as well as the strong development of the CPE during atomization and immediately afterward (see Figure 7a) can be considered proof that large quantities of aerosol droplets were sedimented and that, under certain circumstances, they swept finer particles present in the chamber air along with them down to the bottom, that is to say, finer particles which otherwise might have spread out much more.

Table 4. Virus Aerosol Distribution Tendency

Exposure Time	Sampling Points	No of CPE per Dish	Relative Humidity	Temperature
Prior to atomization (empty control)		ø	50%	21° C
During atomization	I II III	+++	52%	21° C
Immediately after atomization(10 min exposure)	I II III	++	53%	21.1° C
30 min after atomization (10 min exposure)	I II III	35 27 30	51%	21.1° C
1 hr after atomization (10 min exposure)	I II III	22 27 17	59%	21.2° C
1-1/2 hrs after atomization (10 min exposure)	I II III	19 15 14	49%	21.3°C
2 hrs after atomization (10 min exposure)	I II III	10 14 11	50%	21.3°C
2-1/2 hrs after atomization (10 min exposure)	I II III	9 10 4	50%	21.4°C
3 hrs after atomization (10 min exposure)	I II III	5 4 6	50%	21.5°C
3-1/2 hrs after atomization (10 min exposure)	I II III	3 2 ø	50%	21.7°C

Symbols: +++ heavy CPE formation; ++ moderate CPE formation; ø no CPE formation.

In the preliminary experiments we had found that the yield is greater when the cell layers of the plates, which are exposed to the air, are provided with no more than about 1 ml of medium; this was supposed to prevent drying out. After the termination of the experiments, we pipetted 5 ml of preservation medium in (Parker medium 199 and 2% calf serum)(Henneberg and Kohler, 1961). The plates thus charged with medium were placed in a CO_2 container and the latter was placed in an incubator. After 3 days of incubation, the plates were examined for CPE. In these experiments we found that the CPE on those plates, which were exposed 3-1/2 hours after atomization (see Figure 7c), could be read off easily only after the 4th or 5th day. This is why the medium was changed after the 3rd day of incubation in order to preserve the cells.

We might add that plates were exposed in every experiment prior to atomization in order to establish any viruses that might still be present. These plates were called the "empty controls" in the tables. In Table 4, we have the results of these controls.

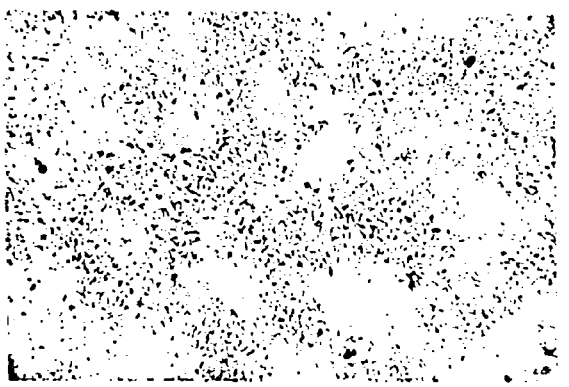

Fig 7a. Abundant CPE formation in plates set up immediately after atomization of virus aerosol.

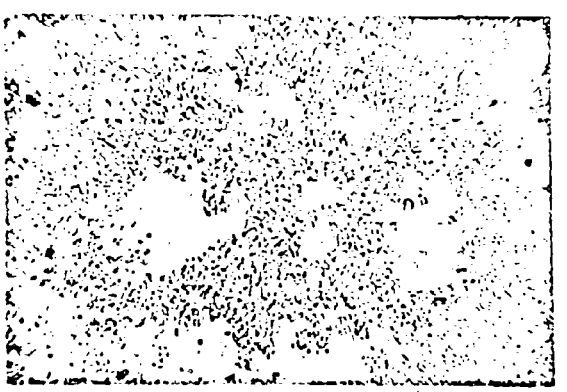

Fig 7b. Half hour after atomization.

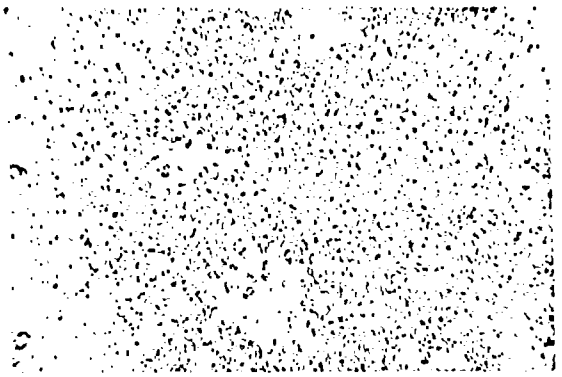

Fig 7c. One hour after atomization.

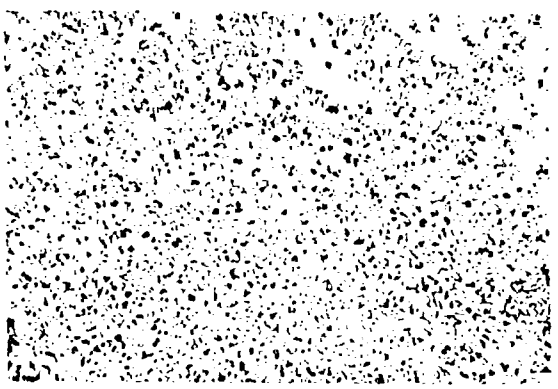

Fig 7d. Two hours after atomization.

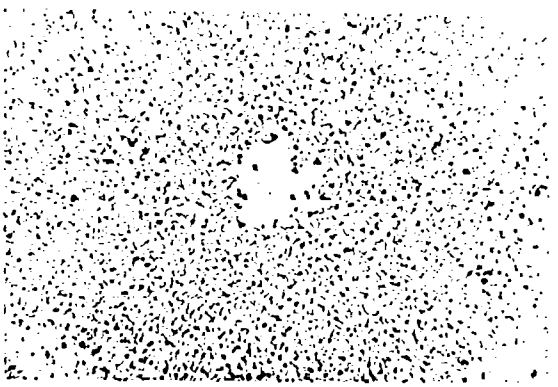

Fig 7e. Three and a half hours after atomization.

(Microscopic photograph, enlarged 1:80)

UV irradiation of the experimentation chamber 24 hours before the start of the experiments, in order to prevent any decontamination, proved to be reliable.

2. Experiment

The suspension time, respectively, the sedimentation tendency of a virus aerosol were now examined at one sampling point in the ch mber, with the rest of the experimental setup remaining the same. The results obtained here are shown in Table 5. Table 5 shows us that no CPE could be read off after 3-1/2 hours. To determine whether viruses could still be found in a relatively long-lasting suspended state 3-1/2 hours after atomization, the air was suctioned off for 5 minutes by means of the aspiration method (about 40-50 liters). Then the washing liquid was thoroughly mixed and a square bottle was inoculated with 1 ml. After 3-4 days of incubation, no CPE could be found in the square bottle. The assertion that all viruses are sedimented after 3-1/2 hours thus would not seem to be correct. Instead it would seem to be more accurate to say that the limit of demonstrability is at 3-1/2 hours after atomization. It is to be assumed that the limit of demonstrability depends on the "infection multiplicity" (that is, the number of infectious virus particles in proportion to the number of available particles). This infection multiplicity decreases in time, so that no more CPE formation is possible.

If we want to examine the sedimentation conditions according to Stokes' law, then we can compute the drop velocity (W) for particles with a diameter of 2.5×10^{-4} cm as follows:

$$W = \frac{2 \cdot r^2 g}{9 \mu} .$$

$r = 1.25 \cdot 10^{-4}$ cm

g = earth's acceleration = 981 cm/s^2
≈ 1 g/cm^3

$\mu = 2 \cdot 10^{-4} \ \frac{g}{cm \cdot sec}$

$W = 61$ cm/hr

If we keep in mind that the experimentation chamber is 51 cm high, this means that the particles were sedimented with a radius of $1.25 \cdot 10^{-4}$ cm within one hour, while the CPE, obtained up to 3-1/2 hours after atomization, was produced by particles of smaller size.

Table 5. Suspension Time and Sedimentation Tendency of Virus Aerosols

Zeit der Exposition (a)	Anzahl der CPE pro Schale (c)	Relative Luftfeuchte (d)	Temperatur (e)
Vor Vernebelung (Leerkontrolle) (f)	0	53%	22°C
Während der Vernebelung (g)	+++	53%	22°C
Unmittelbar nach Vernebelung (h) (10 Min. Exposition)	++	52%	21,8°C
30 Minuten nach Vernebelung (i) (10 Min. Exposition)	38	55%	21,9°C
1 Stunde nach Vernebelung (j) (10 Min. Exposition)	27	54%	22°C
1,5 Stunden nach Vernebelung (k) (10 Min. Exposition)	10	53%	22,3°C
2 Stunden nach Vernebelung (l) (10 Min. Exposition)	12	50%	22,2°C
2,5 Stunden nach Vernebelung (m) (10 Min. Exposition)	9	50%	22,5°C
3 Stunden nach Vernebelung (n) (10 Min. Exposition)	6	50%	22,5°C
3,5 Stunden nach Vernebelung (o) (10 Min. Exposition)	3	49%	22,5°C

Key: [For Items a -- o and Symbols, see Key to Table 4, above.]

3. Experiment

In the experiment described below, we investigated the suspension time of a virus aerosol, with the initial material diluted in this case. Repeated titration revealed values of about $4.8\text{-}5 \cdot 10^6$/ml. The experimental setup was the same.

Table 6 shows the results achieved here. These results indicate that no more viruses could be established 1-1/2 hours after atomization. From this we can conclude that, in the case of an aerosol with reduced titer, the viruses can be established for a shorter period of time only, that is to say, shorter than for the case of an aerosol with a higher titer. The reason for this lies in the fact that the existence of an "infection multiplicity" which would suffice to produce an infection -- a presence which decreases over a period of time -- can be very small, that is to say, very rare in the case of a virus suspension with a low titer.

Table 6. Sedimentation Tendency of a Virus Aerosol with Low Titer

Zeit der Exposition (a)	Anzahl der CPE pro Schale (c)	Relative Luftfeuchte (d)	Temperatur (e)
Vor Vernebelung (Leerkontrolle) (f)	ø	54%	22,2°C
Während der Vernebelung (g)	++	54%	22,3°C
Unmittelbar nach Vernebelung (h) (10 Min. Exposition)	+	53%	22,3°C
30 Minuten nach Vernebelung (i) (10 Min. Exposition)	20	52%	22,3°C
1 Stunde nach Vernebelung (j) (10 Min. Exposition)	11	53%	22,1°C
1,5 Stunden nach Vernebelung (k) (10 Min. Exposition)	8	52%	22°C
2 Stunden nach Vernebelung (l) (10 Min. Exposition)	ø	51%	22°C
2,5 Stunden nach Vernebelung (m) (10 Min. Exposition)	ø	50%	22°C
3 Stunden nach Vernebelung (n) (10 Min. Exposition)	ø	50%	22°C
3,5 Stunden nach Vernebelung (o) (10 Min. Exposition)	ø	40%	22°C

Key: [For Key, see Items a -- o and Symbols, Table 4, above.]

If we consider the "infection multiplicity," then we must figure out the virus content of the droplets which are atomized in a virus suspension of 0,5 ml.

The number of droplets contained in 0,5 ml of atomized virus suspension is computed as follows:

$$X = \frac{V}{V_1}$$

X = Number of droplets
V = Volume of virus suspension atomized
V_1 = Volume of a droplet

The volume of a droplet can be computed according to the following equation (assuming that the droplets are spherical and have a radius of $1.25 \cdot 10^{-4}$):

$$V_1 = \frac{4}{3} \pi r^3 = \frac{4}{3} \cdot 3,14 \cdot 1,25^3 \cdot 10^{-12} \text{ ml}$$

$$V_1 = 8,12 \cdot 10^{-12} \text{ ml}$$

The atomized volume of 0.5 ml virus suspension thus corresponds to a droplet count of:

$$N = \frac{0,5}{8,12 \cdot 10^{-11}} = 0,16 \cdot 10^{10}$$

The number of viruses in 0.5 ml virus suspension was $2.5 \cdot 10^7$ (titer: $5 \cdot 10^7$/ml). This brings us to the question as to the volume of the viruses contained in the droplets:

1. when we have a titer of $5 \cdot 10^7$/ml,
2. in the case of a suspension with the rather low titer of $5 \cdot 10^6$/ml.

In connection with (1), we can say the following:

$$\frac{0,16 \cdot 10^{10}}{2,5 \cdot 10^{7}} = \frac{0,5}{0,25} = 2460$$

that is, that we will have 1 virus in a volume of 2,460 droplets, assuming that the viruses are uniformly distributed.

In connection with (2), above, we can say the following: If we have a titer of $2.5 \cdot 10^6$/0.5 ml, then 1 virus will be contained in 24,600 droplets.

These data tell us something about how great the difference can be in the virus content of the droplets at various titer levels. We can therefore assume with some degree of probability that the short suspension time of particles in a virus suspension with a low titer can be traced to the demonstrability of the "infection multiplicity" which in this case would not be sufficient to cause infection. The above column graph (Figure 3) is intended to illustrate the sedimentation ratios for various titers.

4. Experiment

In this experiment we investigated the influence of the reduced relative humidity (for the method used, see formulas given above). Table 7 shows us the results obtained here. As expected, the viruses revealed a quantitatively small sedimentation tendency when the relative humidity was low while the suspension time remained the same. The virus concentration in the chamber thus decreased more slowly when the humidity was lower and this is probably due to the faster evaporation of the outer envelope of the virus droplets, that is to say, it may be due to the faster shrinkage of their circumference and also of their sedimentation speed.

Table 7. Sedimentation Tendency of Virus Aerosol at Reduced Relative Air Humidity

Zeit der Exposition (a)	Anzahl der CPE pro Schale (c)	Relative Luftfeuchte (d)	Temperatur (e)
Vor Vernebelung (Laborkontrolle) (f)	0	17%	22°C
Während der Vernebelung (g)	+ +	17%	22,2°C
Unmittelbar nach Vernebelung (h) (10 Min. Exposition)	+	17%	22,3°C
30 Minuten nach Vernebelung (i) (10 Min. Exposition)	28	20%	22,2°C
1 Stunde nach Vernebelung (j) (10 Min. Exposition)	20	20%	22,3°C
1,5 Stunden nach Vernebelung (k) (10 Min. Exposition)	16	20%	22,4°C
2 Stunden nach Vernebelung (l) (10 Min. Exposition)	14	20%	22,5°C
2,5 Stunden nach Vernebelung (m) (10 Min. Exposition)	7	18%	22,5°C
3 Stunden nach Vernebelung (n) (10 Min. Exposition)	4	18%	22,6°C
3,5 Stunden nach Vernebelung (10 Min. Exposition) (o)	1	19%	22,6°C

Key: [See Key, Table 4, above]

5. Experiment

Here we wanted to investigate the influence of increased relative humidity on the suspension time of the virus aerosol. The experimental setup was the same. To achieve the desired relative humidity, we sprayed Aqua dest. which had been heated to 20° C, into the chamber from a spray bottle. In this connection we had to make sure that the water droplets necessary for the generation of the air humidity had already been sedimented prior to the subsequent virus atomization. The air humidity was controlled during the experiment with a hygrometer. Table 8 shows us that during the first 30 minutes, when the humidity is high, considerably more virus droplets were sedimented than when the humidity was average or low. During the following interval of time, however, we were able to observe a continual drop in the sedimentation rate. The large number of viruses which were sedimented during the first 30 minutes after atomization caused a rapid reduction in the virus concentration and, as a result of this, also a reduction in the suspension time. This experiment and the preceding experiment show us that humidity plays a very important role in the sedimentation tendency. Figure 3, above, constitutes a graphic illustration of the conditions examined here.

Table 8. Sedimentation Tendency of Virus Aerosol at Increased Relative Air Humidity

Zeit der Exposition (a)	Anzahl der CPE pro Schale (c)	Relative Luftfeuchte (d)	Temperatur (e)
Vor Vernebelung (Leerkontrolle) (f)	0	88%	21°C
Während der Vernebelung (g)	+ + +	90%	21,1°C
Unmittelbar nach Vernebelung (10 Min. Exposition) (h)	+ + +	91%	21,2°C
30 Minuten nach Vernebelung (10 Min. Exposition) (i)	61	89%	21°C
1 Stunde nach Vernebelung (10 Min. Exposition) (j)	24	88%	20,8°C
1,5 Stunden nach Vernebelung (10 Min. Exposition) (k)	17	87%	20,8°C
2 Stunden nach Vernebelung (10 Min. Exposition) (l)	13	85%	20,8°C
2,5 Stunden nach Vernebelung (10 Min. Exposition) (m)	8	80%	20,7°C
3 Stunden nach Vernebelung (10 Min. Exposition) (n)	3	80%	20,7°C
3,5 Stunden nach Vernebelung (10 Min. Exposition) (o)	0	80%	20,7°C

Key: [See Key, Table 4, above]

6. Experiment

Here we investigated the virus count for various heights of chamber air [above the chamber bottom]. For this purpose, the air was suctioned off at heights of 35 cm and 55 cm (see aspiration method). To determine whether air eddies are formed as a result of the fact that the suction bottle is connected in at a higher height level -- air eddies which might have an effect on the results of the bottle suctioning in the lower layer -- we performed a number of control experiments. In two separate experiments, air samples were taken first of all at a height of 35 cm and then, in the other experiment, at a height of 55 cm, 1 hour after atomization. The results obtained here revealed only very minor differences compared to the experiment with the two suction bottles; this means that we were able to prove that the jointly operating suction bottles do not influence each other.

To establish the viruses present in the washing liquid, we removed 1 ml of washing liquid from each suction bottle and we used this quantity to inoculate five square bottles. The count of the CPE in the upper suction bottle revealed average values of 21 viruses per ml. The count of the CPE in the lower suction bottle gave us averages of 58 viruses per ml. This means that the virus count drops as the height increases and the same applies to the infectiousness of the air.

B. Dust Phase

Determination of Dust Particles Infected with T_3 Phages

The vaccine viruses have a relatively high degree of infectiousness and can cause impairment of health in the person conducting the experiments; we therefore selected T_3 phages as experimental models for the next part of our project.

Sedimented, microorganism-containing dust particles can, for instance, stick to wool blankets or to other surfaces in the sick room. By means of various types of handling, such as shaking out or moving blankets, infected fibers can get into the air. Dust particles, with infectious microorganisms sticking to them, can also be rendered capable of suspension as a result of air currents due to the opening or closing of doors. The following part of this study is therefore of particularly great practical importance.

It was interesting to find out here just how long dust particles can be determined in the air and how they behave at varying heights and in varying places throughout a room. In this kind of problem we are not so much interested in determining the size of the particles or in studying the influence of the humidity and the room temperature; likewise, we do not require an atomization of particles with a certain size in studying the problem we are concerned with here.

Material and Methods

The experiment was conducted in a large, blacked-out laboratory room (height: 3.5 m). Dust from a vacuum cleaner was screened several times and was sterilized for 3 hours at a temperature 150-160° C, in dry heat. The sterilized dust was mixed in a Petri dish with a T_3 phage suspension (titer: $4 \cdot 10^8$/ml) and it was then dried for several weeks in an evacuated, $CaCl_2$-containing exsiccator. The dried dust was removed from the surface of the dish and was then ground in a mortar. For atomization, 0.5 g of the infected dust was finely distributed over a large surface and a dust cloud was then produced by means of a pipette which was connected to a compressed-air pump. The change of the concentration of the particles in the room was determined by means of the sedimentation method.

For this purpose we used Petri dishes containing blue-agar; these dishes were inoculated with coli strain "B/am" (bacteria sensitive to T_3 phages).

The dishes were exposed at the following points throughout the room:

1. Middle of the room, floor
2. Right side of room, surface of wash basin
3. Corner of room, 1.5 m below the ceiling on a shelf
4. Left side of room, 1 m above floor on a table
5. Left side of room, corner, 0.5 m above floor
6. Right side of room, corner, on a stool
7. On the door
8. On the floor, under the table
9. Middle of room, 1 m above floor
10. On windown

The exposure times were 1, 2, 3, and 4 hours after atomization; each Petri dish was exposed to the dust cloud for 20 minutes. After termination of the experiment, the closed Petri dishes were kept for 24 hours in an incubator at 37° C and they were then photographed with a camera connected to the plate microscope. The plates "plaques" formed by the T_3 phages and the polymorphia of the dust will be explained in connection with the illustrations.

Another possibility for observation was created with the help of a lamp which generated ray bundles in the darkened room. The processes taking place during this phase were likewise photographed.

Results

I would first of all like to report the findings made throughout the entire experiment by the observer -- provided with headgear, mouth protection, and protective clothing -- with the previously mentioned light source installed to the side.

Prior to atomization, dust particles could be hardly recognized in the light coming in from the side (see Figure 8a). Right after atomization, one could see a thick cloud of particles along the entire length of the light ray and this cloud was distributed in all directions throughout the room after rapid motions (see Figure 8b); 30 minutes after atomization, it was possible to observe a high degree of particle sedimentation (see Figure 8c). As little as 1 hour after atomization, we had an extraordinarily strong concentration decrease and we could observe a uniform distribution of the particles over the entire room (see Figure 8e). One might now expect that the air currents, brought about by the constant movement of the observer in the room as well as by the intentional opening and closing of the door three times, would have caused the dust particles to be distributed unevenly.

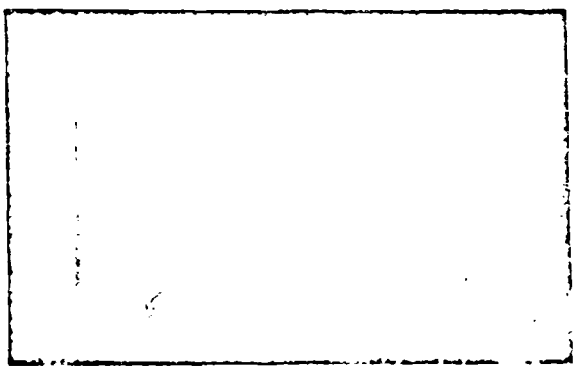

Fig 8a. Condition of experimentation room prior to atomization. Only very few dust particles can be recognized in the picture. (Exposure time: 1/5 second.)

Fig 8b. Movement of dust particles photographed immediately after atomization (Exposure time: 1/5 sec).

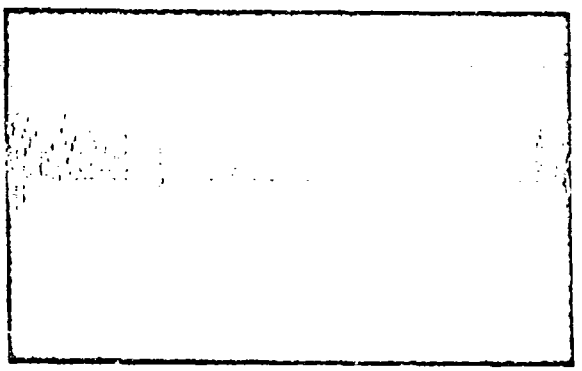

Fig 8c. Increased number of sedimenting dust particles, 30 minutes after atomization (Exposure time: 1/5 sec).

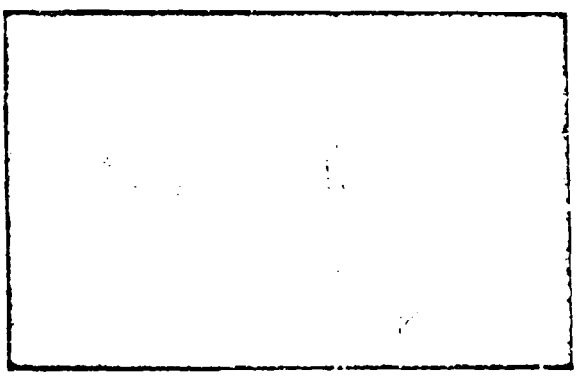

Fig 8d. Photo taken 30 minutes after atomization, 0.5 m below ceiling of room, showing small quantity of dust particles (exposure time: 1/5 sec).

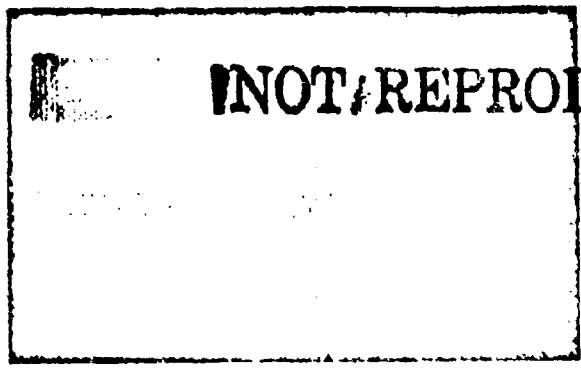

Fig 8a. Photo taken 1 hour after atomization, 1.5 m below room ceiling, showing completely uniform distribution of dust particles (exposure time: 1/5 sec).

The observations made at various height levels throughout the room revealed the following:

At 30 minutes after atomization, the dust volume 0.5 m below the ceiling of the room was at a minimum (see Figure 8d); this can be explained by the abovementioned heavy sedimentation; 1 hour after atomization, a smaller number of dust particles was observed 0.5 m below the ceiling than 1.5 m below the room ceiling (see Fig 8e). This can probably be explained by saying that the largest quantity of dust particles at that time is already in sedimentation; 90 minutes after atomization, the situation was exactly opposite of what it was earlier, 0.5 m below the room ceiling, that is to say, that there was a relative increase in the quantity of the particles as the height increased. This phenomenon can be explained by saying that the uniform distribution of the dust particles, which had already begun at that moment, enabled the dust to continue to be suspended in the room, in the air, in spite of the laws of gravity because the particle number in the volume unit is greater in the lower layers than in the higher layers and because an air volume streaming upward from below will move more particles to the higher levels than an equally large volume of air, coming from above, can carry downward.

The results achieved with phages in this experiment are shown in Table 9. The table shows that there was a very rapid drop in the dust concentration 1 hour after atomization. The quantity of the phages that had dropped into the dishes was just about uniform in all phages; 2 hours after atomization only very few phages could be established and 3 hours after atomization the number of phages that could still be determined had dropped to a minimum; 4 hours after atomization, none of the dishes exposed revealed any phages.

Table 9. Sedimentation Tendency of Dust Particles Infected With T_3 Phages in a Laboratory Room

Expositionsstellen der Petrischalen[1] (a)	(b) Anzahl der Phagen 1 Std. nach Verstäubung (c)	2 Std. nach Verstäubung (d)	3 Std. nach Verstäubung (e)	4 Std. nach Verstäubung (f)
1.	+	4	2	ø
2.	+	1	1	ø
3.	+	1	ø	ø
4.	+	5	2	ø
5.	+	4	2	ø
6.	+	4	3	ø
7.	+	2	1	ø
8.	+	4	1	ø
9.	+	2	1	ø
10.	+	1	ø	ø

Key: a. Places at which Petri dishes were exposed (see Fig 8e)
b. Number of phages
c. One hour after atomization
d. Two hours after atomization
e. Three hours after atomization
f. Four hours after atomization

+ Number of phages exceeded the limit of countability
ø No phages could be established here

It is interesting to note that the findings made with the help of the incoming light, as shown in the photos, agreed completely with the results obtained on the basis of the observations of phage growth on the exposed Petri dishes.

Discussion

Diseases such as smallpox or diseases of the respiratory tract, measles, or varicella can be caused also by the fact that the viruses reach the human organism by means of aspiration through the respiratory tracts. In this kind of infection, the suspension time of the microorganism-bearing particles in the air constitutes an important factor. In order to determine how aerogenic viruses can be communicated and can thus cause infections, we must learn more about the physical magnitudes which enable the particles to remain in suspension. This kind of suspension state assumes particular importance in the dust particles because the latter have a large surface and are quite bulky, although their mass or weight might be relatively smaller.

If the droplet nuclei (see Note)(Gordon and Ingalls, 1946) are sedimented, then they may get into the air again together with dust particles or they may stick to these dust particles (air-borne or dust-borne infection)(see Note)(Gordon and Ingalls, 1964).

(Note) These are factors which are often mentioned in British and American literature but for which we do not yet have any definitive terms in the German language.)

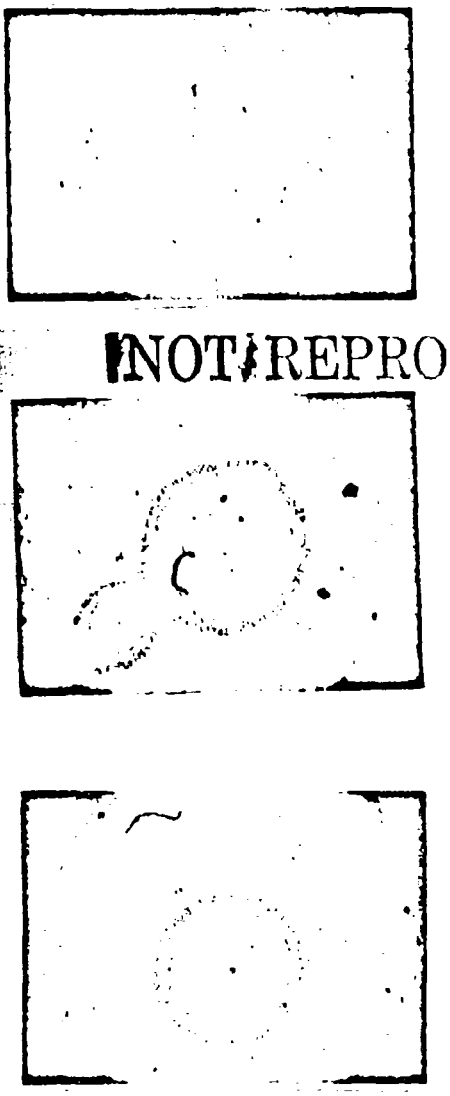

Fig 9a, b, c. Plaques of dust particles charged with T_3 phages. The photos show the polymorphic aspect of the dust, dust particles inside the plaques, as well as those that did not produce any plaques. (Microscopic photos, enlarged 1:33)

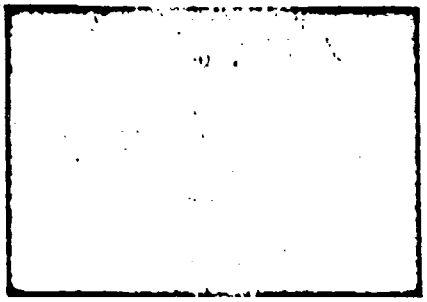

Fig 9d. Negative result after 4 hours.

The so-called "dust-borne" infection phase applies in viruses which remain on dust particles for a long time; this is true of the viruses of smallpox, psittacosis, poliomyelitis, influenza, and measles (Schwietzke, 1961).

The data developed experimentally in Section B, above, reveal that dust particles charged with T_3 phages are sedimented considerably faster than was the case in the T_3 droplet phase (considering the amount of the volume and the environmental conditions of the experimentation chamber, on the one hand, and the identical conditions in the laboratory room in which the dust phase experiments were conducted, on the other hand). We can explain this as follows: the T_3 phages are probably mostly connected with coarse-dispersed dust particles. This is supported by the rather striking decrease in the aerosol concentration due to the sedimentation of the large dust particles during the first 30 minutes after atomization. However, this may lead to the possibility that a certain bacteria volume might not be connected with the dust particles (see Figure 9a, b, c) or that the bacteria lost this contact or connection at the time of atomization — which, in turn, meant that individual T_3 phages present in the air could not be spotted. If we were correct in assuming that the phages lost their contact with the dust particles during atomization, then the time of their stay in the air would be the same or it even exceeded the time involved in the droplet phase because we found salts and other substances on the surface of the T_3 phages in the droplet phase after the complete evaporation of the outside water envelope; these salts and other substances had been present in the drop and thus caused a faster sedimentation to take place.

Let us now correlate the experimental results with the factors that enter the picture in the sedimentation of aerosols.

The suspension time is determined by the following factors:

1. Shape and Size of Particles

(a) Shape of Particles

It is assumed that the particles are spherical and this is probably quite correct, with some degree of probability of course.

(b) Particle Size

This factor is very important for the duration of suspension and for other events which will be mentioned later on; this factor, in turn, depends on other forces:

One very important factor which is yet to be studied theoretically is the coagulation of particles as a result of Brown's movement.

In Russian literature on the subject (Bolotovskiy, 1961), we can find a formula which tells us something about the coagulation of the particles as a result of Brown's movement. The solution of this formula calls for the average diameter of the droplets produced by the nozzle. Unfortunately, we do not know the exact average value for the particles (see Material and Methodology used in Preliminary Experiments).

Furthermore, Shishkin (1954) showed that, if we have a particle diameter of 2 mu, there is a decrease in the number of particles due to the so-called "Brown coagulation"; this decrease amounts to 50% within 70 hours. If we keep in mind the theory developed by Remy (1949) -- to the effect that the extreme size which the particles may have can be expressed in terms of a diameter of $2.5 \cdot 10^{-4}$ cm, and if we consider information supplied by the Drager Company according to which the average of the nozzle-generated droplets does not exceed a diameter of $2 \cdot 10^{-4}$ -- then we can say that the coagulation of the particles on the basis of Brown's movement has no significance at all.

One factor, which in actual practice corresponds to the effect of the drafts in rooms and corridors, is called the turbulent coagulation (Bolotovskiy, 1961). By this we mean a rather forceful coagulation which is the result of uneven air movements and which can cause a change in the particle size. This form of coagulation, however, need not be taken into consideration in Section A because its effect extends only over a rather short interval of time during atomization and because such uneven air movements can be avoided if we proceed cautiously in handling things in the chamber or room. A collision of the particles on the basis of the differences in the drop velocity can be ruled out according to the computations of Langmuir (1948).

One extremely important factor is the coagulation of the particles on the basis of their electrical charge (Bolotovskiy, 1961). There is nothing we can say about the effect of electrical coagulation because the electrical charge of the particles was not investigated. The assumption that we get unipolarity if we artificially produce aerosols can just about be ruled out. But we can assume with some degree of certainty that the suspended particles are charged after a certain time even when we use artificial or synthetic aerosols; they are charged here when they adsorb gas ions from the air. The charge process here can be understood as a process of diffusion of the small ions into the particle (Kuhn, 1948). In this connection the particles take on a boundary charge which depends only to a very minor extent on the level of the original ion density. If this were the case, then one would have to anticipate a change in the particle size.

2. Relative Humidity

Our experiments showed that relative humidity has a great effect on the suspension time of the particles. As a result of the experiments we can say that a high virus concentration is preserved longest when the air humidity is low; this is due to the faster evaporation of the outer layer of the virus-containing droplets, that is to say, the faster reduction of their circumference and thus also the faster reduction of their sedimentation speed. As the humidity increases, the speed of evaporation decreases and the droplets are sedimented faster.

The size of the particles influences not only the suspension time but is also critical with respect to the places throughout the respiratory tract where these particles are deposited. According to Noble, et al (1963) the diameter of human-pathogenic suspended particles is between 4 mu and 20 mu. The following data are available with respect to the deposit of a polydispersed aerosol in various parts of the animal respiratory system:

In rabbit experiments (Buckland, et al, 1950), it was found that, when an aerosol with a particle radius of 0.5 mu is inhaled, 29% of the particles are deposited in the nasopharynx, 13-19% are deposited in the windpipes, and 51-58% are deposited in the pulmonary alveoli. Particles with a radius of 2 mu could be determined in the nasopharynx to the extent of 65% and those with a radius of 4 mu were found to the extent of 98%.

Mouse experiments (Shoshkes, 1950) revealed the following particle distribution over the bronchia, the bronchial alveoli, and the pulmonary aoveoli: particles with a radius of 0.2-0.62 mu were found to the extent of 26%, 32% and 42% and those with a radius of 1.46-1.88 mu were found to the extent of 48%, respectively, 15%.

On this basis, we can conclude that the inhaled particles of the virus suspension will penetrate all the more deeply into the respiratory tract, the smaller their circumference happens to be.

3. Virus Content of a Droplet

In the experiments we found that viruses can be established in an aerosol with a higher titer for a longer time than in an aerosol with a lower titer. If we want to determine the virus content of a droplet when we have a certain titer, then we must first of all compute the volume of a droplet; this can be done with the help of the formula given earlier.

Explanation:

(a) This probably has something to do with the "infection multiplicity," that is to say, with the number of viruses necessary to trigger a cytopathogenic effect. This means that, if we work with aerosols with a high titer, there must be a possibility for a greater "infection multiplicity" than in the case of aerosols with a low titer.

(b) The probability of demonstrability tends to decrease over a period of time to a much greater extent when we have a low titer than when we have a high titer.

We might mention the following places where infectious aerosols can be generated and from which they might spread in actual practice.

(a) First of all we have the hospital environment here where the droplets get into the air whenever patients or other virus carriers sneeze, cough, or talk. Here the air, as the carrier of the viruses, plays an important role in the vicious circle of hospitalism (Henneberg, 1963). This is why it is extraordinarily important to educate and inform all persons involved (among others, Henneberg, 1961). The fact that the communication of hepatitis, which currently is probably the most frequent disease encountered under hospitalism, can take place also because droplets separated by patients get into the air and perhaps come to adhere to some of the routine instruments used in the hospital (probes, tweezers, catheters, syringes, etc), fully confirms Henneberg's attitude toward this problem also with respect to the importance of educating and informing everyone on this topic.

Finally we might mention here the prevention of aerogenically communicated infectious diseases in pediatric clinics. Here it is very important to set up individual cubicles with no more than 2 beds and to see to the decontamination of the air in rooms through UV irradiation, the elimination of any other communication possibilities such as door knobs, water faucets, etc, through the introduction of elbow-operated or foot-operated fixtures and devices. In 1965, for instance, I found out that the infectious disease department of the Berlin-Charlottenburg Municipal Children's Clinic (Prof Dr Wiesener, Clinic Director) was able to achieve considerable successes here.

(b) Infectious aerosols can originate in many ways in the laboratories (Albrecht, 1961). Just about any work operation or activity can lead to the formation of an aerosol which may under certain circumstances be infectious. Here the source of origin of this aerosol in most cases is not far from the nose and the mouth of the persons working in the laboratory. This mostly involves a polydispersed aerosol which can develop whenever rapidly moved liquids are suddenly braked very severely when they encounter an obstacle and when these liquids are turned into a form of spray as a result of this. Generally, we only have very few particles with the kind of size that would cause them to remain in suspension even if the air were calm. The virus-carrying particles become capable of suspension in the following way: either the liquid made up of larger particles is quickly evaporated in the dry laboratory air or the viruses, sprayed over the surfaces, are carried into the air after drying, together with dust particles or in a situation in which they stick to these dust particles ("air-borne," respectively, "dust-borne infection").

Infectious aerosols can develop in connection with the following operations in the laboratory:

1. When we mix virus-containing liquids by means of pipettes or when these liquids are blown out of the pipettes, we get an aerosol which not infrequently consists of numerous droplets.

2. If we use an annealed metal loop for inoculation or for other handling of cultures, then we might tear the liquid film in that loop if we move it rapidly through the air and we can thus infect the air. Furthermore, we can get aerosols in the annealing of loops, when we submerge hot loops in liquids or due to the vibration of loops, for instance, when we spread cultures on slides.

3. When liquid germ or virus cultures are moved forcefully and abruptly in agitators, mixers, fermenters, syringes, or as a result of agitators or ultrasound or perhaps in centrifuges, we can also get aerosols. If the culture containers are closed off, then the aerosol will not escape until the container is opened.

4. When we work with mortars and similar instruments to grind up solid virus cultures or infected materials we can likewise form an aerosol.

5. There is furthermore a possibility that the microorganisms might escape into the surrounding room air in connection with the dry-freezing method, both during drying as such and during the opening of the glass vessels which are under a vacuum.

(c) Infected experimental animals in the animal stable (for instance, psittacosis, spotted fever) constitute a danger source of the first order. The elimination of the animals causes the stable air to be enriched with virus-containing droplets, respectively, infectious dust particles. The animals keep moving around constantly and they therefore keep the dust, infected by their excrements, constantly stirred up and in motion.

It is extremely difficult to answer the question as to whether aerogenic virus communication is possible in the open terrain, for instance, when the windows of a hospital room are opened or when we air out laboratory rooms in which we work with infectious materials; likewise, it is difficult to say whether the already experimentally established anemochoria (Weltzien) (aerogenous communication of plant-pathogenic fungi) also applies to viruses. If we look at the conditions under which viruses are transmitted in enclosed areas or rooms, then there is much more of a probability of aerogenic infection as the suspension time of the particles increases. The suspension time again, depends upon the air movement, that is to say, if the air movement is uniform, the particles remain suspended longer. If the air is stirred up, then the aerosols are sedimented faster and they are then converted into aerogels. They stick to the surfaces until they get back into the air the next time the air is stirred up.

The situation however is entirely different in the open terrain. Here practically all air currents produce eddies which are caused either by boundary layer drift or by obstacles (houses, rooms, etc). This phenomenon accordingly can extensively cancel out the effect of the laws of gravity with respect to a dust particle in the atmosphere. Of course, the altitude of particle flight can be practically unlimited here. The dilution of the concentration of particles, developing here, increases as the flying altitude increases and we can therefore assume that we will have a decrease in the probability of infection in the open terrain. On the basis

of these theoretical possibilities we might say that the possibility of virus communication in the open terrain (for instance, near hospitals) would seem to be extremely small. Of course, we must not overlook the resistance of some virus types on [to] dust because it would at any rate be possible that this property might promote a transmission of the particular viruses under certain circumstances.

Compared to the study of the aerogenous communication of viruses in enclosed areas, an investigation of virus transmission in the open terrain would encounter by far greater difficulties -- to begin with, in regard to the possibility of identifying and determining the presence of these viruses.

Summary

In order to determine the laws of the propagation of viruses by aerosols (dust, droplets), experiments on particle sedimentation were made under various conditions involving particle size, relative air humidity, and air temperature. As model viruses we used T_3 bacteriophages because the demonstrability is rather easily achieved here and we used vaccine viruses because these are particularly interesting under our conditions. A number of methods had to be tested in order to establish the presence of viruses in the air. It was possible to identify viruses, which were sedimented with dust or droplets, up to 3-1/2 hours. Conclusions as to the propagation of viruses are drawn on the basis of the experimental results.

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